

DRUG DISCOVERY

GC-MS analysis of *Ficus religiosa* root extract and its effect against periodontal diseaseEswaralakshmi R^{1*}, Arifa Khatoun²

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ABSTRACT

F. religiosa is a sacred tree native to India where it grows up to elevations of 5,000 ft (1,524 m) (Neal 1948). It is known to be a sacred plant in India and since ancient times it is widely being used to treat various ailments like skin diseases, heart diseases, constipation, dysentery, snakebite and important constituent of various traditional herbal preparations like shankha vati, chandraprabha vati, kaminivindravan rasa. In the present investigations GC-MS analysis was carried out to quantify the different compounds present in the root extract of *Ficus religiosa*. The results shows 25 compounds are present in the root extract of *Ficus religiosa*. The antibacterial activity root extract was tested against most pathogenic periodontal disease causing bacteria, *Streptococcus mutans*. Disc diffusion method was used to study antibacterial effect. The results show its effect against *Streptococcus mutans*. GC-MS Analysis results shows that it has certain compounds that have antibacterial and antiviral activity.

Keywords: *Ficus religiosa*; Antibacterial activity; *Streptococcus Mutans*; Periodontal disease; Ethanollic Extract.

Abbreviations: GC-MS - Gas Chromatography-Mass Spectrometry; VOCs - Volatile Organic Compounds; HIV - Human Immunodeficiency Virus; NCTC - National Collection of Types Cultures; NSC - Neural Stem Cultures; NCIMB - National Collection of Industrial and Marine Bacteria; L.B - Luria Bertani.

1. INTRODUCTION

Dental plaque, an integral part of the healthy human oral cavity, can become the centre of pathological developments, leading to various dental and periodontal diseases (Hirsch and Clarke, 1989) (Fig.1). More than 75% of adults all over the world have some form of gum disease but most are unaware of it. While gingivitis is nearly universal among children and adolescents, Periodontitis typically occurs as people get older and is most common after age 35. Periodontal disease often occurs in members of the same family. Genetic factors may play a role. Also the bacteria that cause periodontal disease may be able to pass to others through saliva. *Streptococcus mutans* is a Gram-positive bacterium that lives in the mouth. It can thrive in temperature ranging from 18-40°C. It metabolizes different kinds of carbohydrates, creating acidic environment in the mouth as a result of this process. This acidic environment in the mouth is what causes the tooth decay. It is the leading

Dental plaque:

Dental plaque is a film of mucus and bacteria deposited on the teeth that encourages the development of dental caries

Periodontium:

Periodontium is the tissues investing and supporting the teeth, including the cementum, periodontal ligament, alveolar bone, and gingiva.

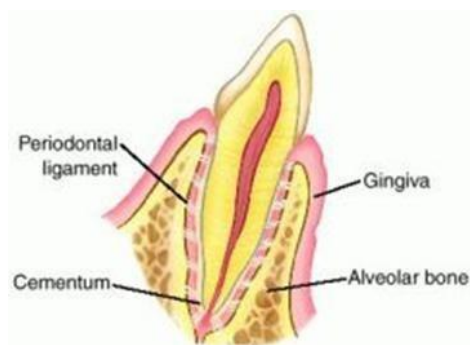


Figure 1

Tissues of Periodontium

cause of dental caries (tooth decay) worldwide. *S. mutans* is considered to be the most carcinogenic of all of the oral *Streptococci*. Peepal (Arasa maram, Tamil) is a large, fast growing deciduous tree. It has a heart shaped leaves (Fig.2). It is a medium size tree and has a large crown with the

Periodontitis:
Periodontitis is a disease of the periodontium characterized by inflammation of the gums, resorption of the alveolar bone, and degeneration of the periodontal membrane.

Dyspepsia:
It is known as upset stomach or indigestion, refers to a condition of impaired digestion.



Figure 2

Ficus religiosa (Peepal Tree)

wonderful wide spreading branches. The whole plant or its specific parts (leaves, stem, and roots) are known to have medicinal properties and have a long history of use by indigenous and tribal people in India (Deepika et al., 2011). *Ficus religiosa* is used as an Ayurvedic medicine in India and Unani medicine in Arab countries for the treatment of diabetes, stress, dyspepsia, abdominal pain, inflammation, Jaundice,

filter paper. Then the filtrate was collected and centrifuged. The centrifuged sample was allowed to evaporate for future use. Fresh root extract was also prepared by the same procedure.

2.3. Anti Microbial Test

2.3.1. Collection of Bacterial culture

Bacterial strain was collected from Madras University, Guindy Campus and incubated at 37°C. A loopful of bacterial strain was added to a 50ml of L.B. broth in conical flask and it was incubated at 37°C for overnight.

2.3.2. Agar well diffusion method

Agar well diffusion method was used to detect the microbial activity of root extract against the above mentioned bacteria. The culture of bacteria was spread on to the agar plates using L rod. The wells were cut using gel puncture. Different concentrations of the plant extracts were added to the wells. The plates were incubated and zone of bacterial inhibition was measured (Sharma, 1981). Doxycycline 100 mg is taken as control. This tablet is dissolved in 10 ml distilled water and control was prepared. This experiment was performed by the method of (Bauer et al., 1966). The bacteria was inoculated in brain heart infusion medium (BHI) and well diffusion method was performed. In the wells the root extract, control (Doxycycline) and ethanol was added

AGAR WELL DIFFUSION METHOD

The inoculation of microorganism was prepared from bacterial culture. About 15 to 20 ml of Muller-Hinton agar medium was poured in the sterilized Petri dishes and allowed to solidify. One drop of inoculum was spread over the medium by a rod. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. Different concentrations of the plant extracts were added to the wells. Plates were incubated in air at 37°C for 24 h. Antimicrobial activities were evaluated by measuring the inhibition zone diameters.

DOXYCYCLINE

It is an antibiotic used to treat aggressive periodontal disease. It is used in the form of gel. It works by preventing the growth and spread of bacteria. This antibiotic will not work for colds, flu, or other viral infections. It causes side effects like diarrhea, itching of the rectum or vagina, sore mouth etc.

enlargement of spleen and congestive heart failure. Despite the pharmacological activities described, no attempt has been made to determine the antibacterial activity of the root extract of *F. religiosa*. Thus, the present study was initiated to evaluate the antibacterial activity of ethanol extract of the root of *F. religiosa* and to estimate the Novel compounds present in *Ficus religiosa* root by GC-MS analysis.

2. MATERIALS AND METHODS

2.1. Sample Collection

Root sample of *Ficus religiosa* was collected from Sipkot, Chennai and shade dried. Fresh roots were collected prior to the experiment.

2.2. Root Extract Preparation

Ethanol extract of the root is prepared by soaking 1gram of shade dried roots with 10ml of ethanol. The roots were left in ethanol for 72 hours and then grinded with the help of mortar and pestle. Then it is filtered through Whattmann no.1

separately in each well in the concentration ranging from 20µl, 40µl, 60µl 80µl and 100µl. After 24 hours of incubation zone of inhibition was measured.

2.4 GC-MS (Gas Chromatography-Mass Spectrometry)

GC/MS is a technique that can be used to separate volatile organic compounds (VOCs). We can combine GC or MS with other separation and analytical techniques. The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of known mass spectra, covering several thousand compounds, is stored on a computer. Mass

GC-MS:

An abbreviation for the instrumental technique which couples the powerful separation potential of gas chromatography with the specific characterization ability of mass spectroscopy.

spectrometry is considered the only definitive analytical detector.

The purified sample was used for GC/MS to analyze the compounds present in the sample.

Conditions

Column Oven Temperature: 70°C
 Injector Temperature : 200°C
 Injection Mode : split
 Split Ratio : 40
 Flow Control Mode : Linear Velocity

Column Flow : 1.51ml/min
 Carrier Gas : Helium 99.9995%
 purity

Column Oven Temperature Program

Rate	Temperature	Hold-Time (minute)
-	70	2
10	300	10 (35.0 min)

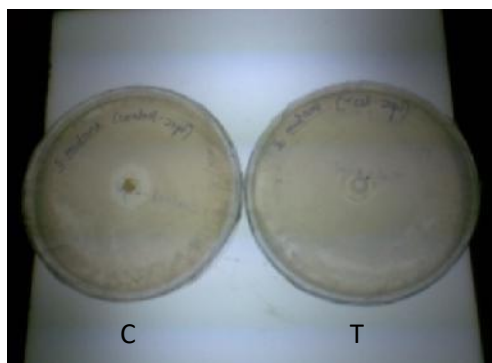


Figure (3a)

20µl

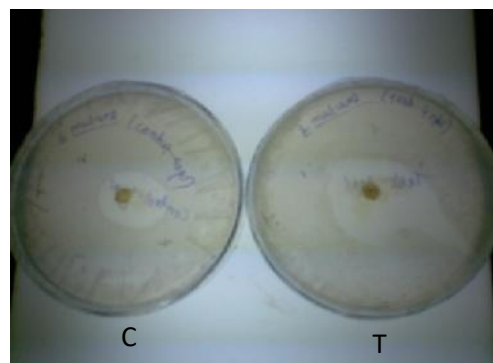


Figure (3b)

40 µl

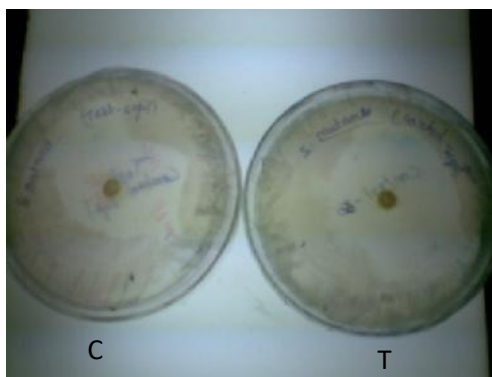


Figure (3c)

60 µl

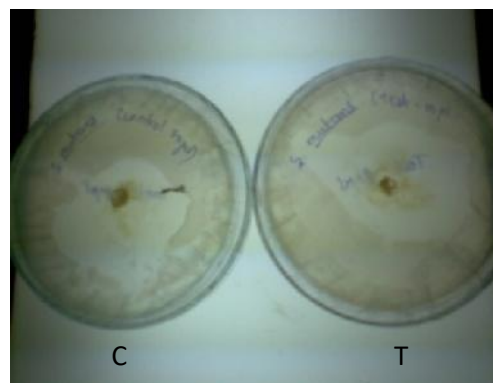


Figure (3d)

80 µl

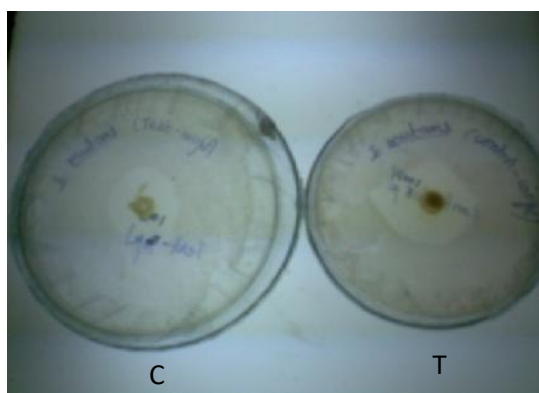


Figure (3e)

100 µl

Figure 3

Antibacterial activity of *Ficus religiosa* root extract against *Streptococcus mutans*

Analgesic:

An analgesic (also known as a painkiller) is any member of the group of drugs used to relieve pain.

3. RESULTS AND DISCUSSIONS

3.1. Antibacterial activity

Ficus religiosa root extract shows zone of inhibition at 20 μ l is 5 mm (Fig.3a), 40 μ l is 15 mm (Fig.3b), 60 μ l is 22 mm (Fig.3c), 80 μ l is 26mm (Fig.3d) and 100 μ l is 18 mm (Fig.3e), (Table 1). Doxycycline is a tablet that is used to control periodontal disease. It is evident that 80 μ l extract shows 26mm zone of inhibition which or less similar to control. Hence, it is evident that

antibacterial effect of *Ficus religiosa* root extract is more efficient like control. *Ficus religiosa* root extract shows antimicrobial activity against *Staphyococcus aureus*, *E. coli* and *Klebsiella pneumonia* (Murthy et al., 2011). Leaf decoction has been used as an analgesic for toothache (Ripu and Rainer, 2006).

3.2. GC-MS analysis

The root extract prepared was analyzed for the presence of phytochemical components using GC-MS. The result shows that 25 different compounds were present in it (Fig.4). Among the 25 compounds following compounds shows high peak value a) trans-O-Dithiane-4,5-diol, b) 1,3,4,5-Tetrahydroxy-Cyclohexane Carboxylic acid, c) Stimast-5-en-3-ol, d) Lup-20(29)-en-3yl acetate, e) Methyl Commate C, d) Acetic acid, 17-(1,5-dimethylhex-4-enyl)-4,4,8,10,14-pentamethyl-2,3,4,5,6,7,8,9.

The human immunodeficiency virus type 1 (HIV-1) nucleocapsid p7 protein contains two retrovirus-type zinc finger domains that are required for multiple phases of viral replication. Through an extensive drug discovery program of the National Cancer Institute, a non-dissociable tethered dithiane compound (1, 2-dithiane-4, 5-diol, 1, 1-dioxide, *cis*; NSC 624151) has been identified. This compound specifically attacks the retroviral zinc fingers, but not other antiviral targets. The lead compound demonstrated broad antiretroviral activity, ranging from field isolates and drug-resistant strains of HIV-1 to HIV-2 and simian immunodeficiency virus. The compound directly inactivated HIV-1 virions and blocked production of infectious virus from cells harboring integrated proviral DNA. NSC 624151 provides a scaffold from which medicinal chemists can develop novel compounds for the therapeutic treatment of HIV infection (William G. Rice et al., 1997).

Stigmast-5-en-3 β -ol(β -Sitosterol) was isolated and characterized from chloroform extract of *H. spinosa* leaves and this is a phytosterol. β -Sitosterol reduce carcinogen-induced cancer of the colon. It shows antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects and inhibition of spermatogenesis. Beta-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have

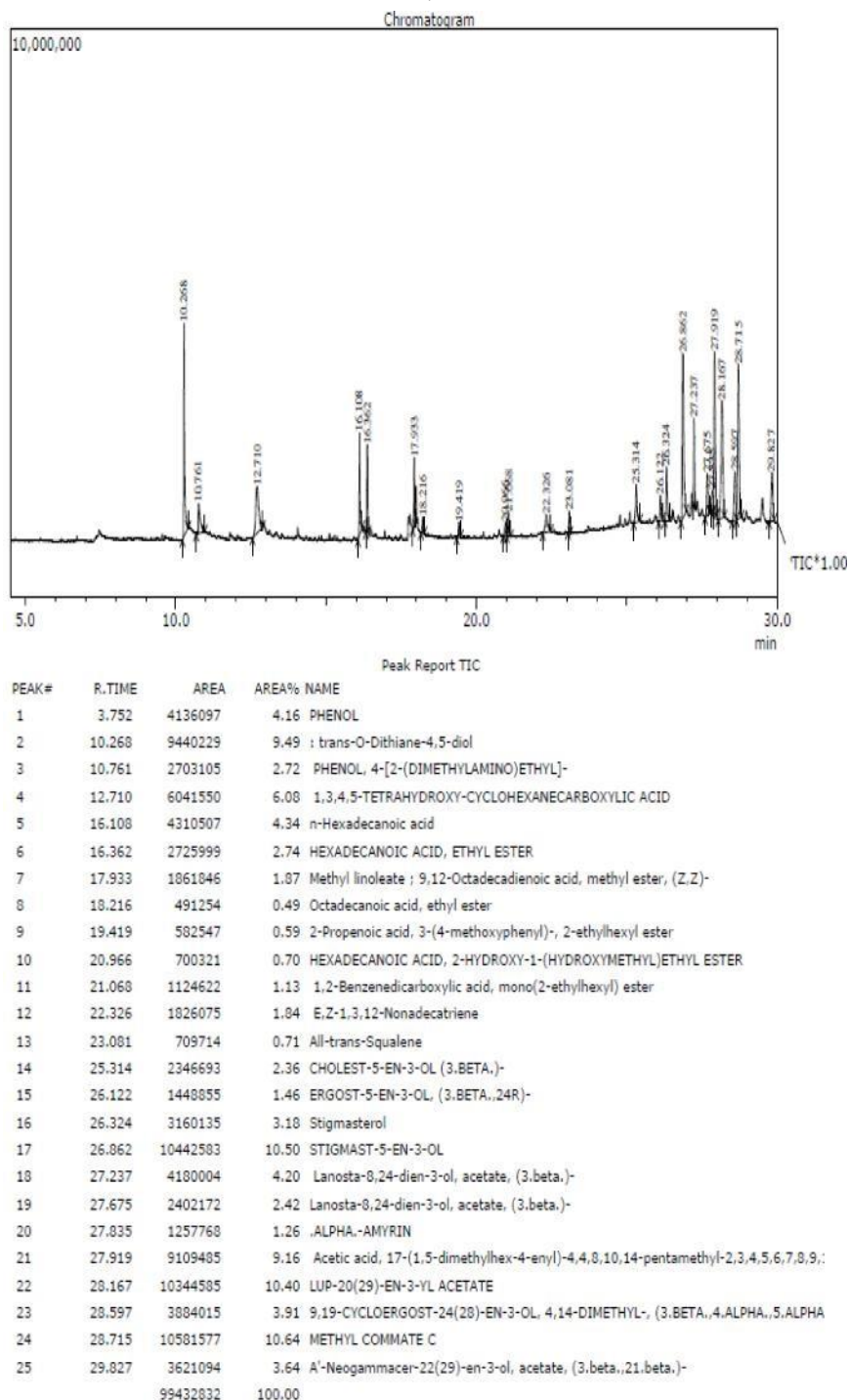


Figure 4

GC-MS analysis of *Ficus religiosa* root extract

other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity (Arjun Patra et al., 2010).

Table 1 Antibacterial activity of *Ficus religiosa* root extract against *Streptococcus mutans*

S. No.	Concentration	Root extract	Control
1	20 µl	5 mm	18 mm
2	40 µl	15 mm	28 mm
3	60 µl	22 mm	32 mm
4	80 µl	26 mm	27 mm
5	100 µl	18 mm	23 mm

Methyl commate c isolated from *Commiphora glandulosa* Schinz exhibited good *in vitro* antibacterial activity against gram positive bacteria (*Bacillus subtilis*: NCTC 10073), (*Clostridium perfringens*: NCTC 8237), (*Staphylococcus aureus*: NCIMB 9518) and also drug resistant strains (*S.aureus*:XU212-tetracycline resistant strain), (*S. aureus* SA1199B-norfloxacin resistant strain) (Daniel Mothanka et al., 2010).

The compounds of the chloroform extract were betuline (Lup-20(29)-ene-3 [128-diol], (306)-l 2-oleanen-3-yl-acetate, 3β-lup-20(29)-en-3-ol (lupeol), β-amyrin (3β-olean-12-en-3-ol) and 6, 10, 14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone). It has become clear that chloroform and ethanol extracts of *O. caricum* has a potential to inhibit the growth of multi-resistant *S. maftophifia*, *S. aureus* and some staphylococci. Hence, the extracts of *O. caricum* may be useful as alternative antimicrobial agents for multi-antibiotic resistant bacteria (Aysel ugur et al., 2011).

Acetic acid, 17-(1,5-dimethylhex-4-enyl)-

4,4,8,10,14-pentamethyl-2,3,4,5,6,7,8,9 is also known as quinic acid. It shows anti-hepatitis B virus activity and capability to inhibit type I HIV.

The pharmaceutical composition and bioactive component may be used to enhance immune competency, treat disorders associated with the immune system, inhibit the inflammatory response, treat disorders associated with the inflammatory response, enhance the anti-tumor response, and treat disorders associated with the response to tumor formation and growth, all in mammals.

4. CONCLUSION

The antimicrobial property of the *Ficus religiosa* against *S. Mutans* and previous studies carried out in this plant establishes their efficacy as in claimed in Siddha literature and previous ethnobotanical studies. It suggests that it is a potential anti-bacterial drug against periodontal disease causing bacteria's. GC-MS analysis shows that this tree has 25 phytochemical components in its root. The compounds like Lup-20(29)-en-3yl Acetate, Methyl Commate C present in the root extract act as a good antimicrobial agent. Hence, the extract of *Ficus religiosa* may be useful as an alternative agent for multi antibiotic resistant bacteria. Among all the GC-MS compounds, trans-o-Dithiane, 4-5, Diol, shows multiple inhibitory effects against HIV type- I nucleocapsid.

SUMMARY OF RESEARCH

1. *Ficus religiosa*, a medicinal tree has been selected for the present study to evaluate the antibacterial activity against most pathogenic periodontal disease causing bacteria, *Streptococcus mutans*.
2. In this study, it has become clear that ethanol extract of *Ficus religiosa* has great potential to inhibit the growth of predominant microbes present in the mouth that cause periodontal disease.
3. Hence, the extracts of *Ficus religiosa* may be useful as alternative antimicrobial agents for multi- antibiotic resistant bacteria.
4. Gc-MS analysis also proves *Ficus religiosa* contain compounds like LUP-20(29)-EN-3YL ACETATE, METHYL COMMATE C which act as good antimicrobial agent.
5. Trans-O-Dithiane-4,5-diol compound present in the root extract shows multiple inhibitory effects against HIV type-I nucleocapsid7.

FUTURE ISSUES

1. In extraction of trans-o-dithiane, is any other compound present in the root extract?
2. What is the effect of trans-o-dithiane and Acetic acid,17-(1,5-dimethylhex-4-enyl)-4,4,8,10,14-pentamethyl-2,3,4,5,6,7,8,9 compound against viruses?
3. How potent it help to control HIV?

DISCLOSURE STATEMENT

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